

**A TAXONOMIC STUDY OF THE GENUS *LAURENCIA*
(CERAMIALES, RHODOPHYTA) FROM VIETNAM.
II. *LAURENCIA LAGENIFORMIS* SP. NOV.**

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ABSTRACT — *Laurencia lageniformis* Masuda (Ceramiales, Rhodophyta) from Vietnam is described as a new species. It is characterised by the following set of morphological features: 1) mostly distichous-opposite branching; 2) the production of four periaxial cells from each axial cell; 3) the presence of longitudinally oriented secondary pit-connection between contiguous superficial cortical cells; 4) non-projecting superficial cortical cells, even at the apices of branches; 5) superficial cortical cells that slightly change in size from branch apices to more proximal positions; 6) the presence of lenticular thickenings in the walls of medullary cells; 7) a parallel arrangement of tetrasporangia; and 8) flask-shaped cystocarps. This species is also characterised by the production of the C15 acetogenin 12-*epi*-obtusenyne and the diterpenoid deoxyparguerol 16-acetate (15-bromo-2,16-diacetoxy-7-hydroxy-9(11)-parguerene) as halogenated secondary metabolites.

RÉSUMÉ — *Laurencia lageniformis* Masuda (Ceramiales, Rhodophyta), récoltée au Vietnam, est décrite comme espèce nouvelle. Cette espèce se caractérise par l'ensemble de caractéristiques suivantes: 1) ramification essentiellement opposée-distique; 2) production de quatre cellules périaxiales à partir de chaque cellule axiale; 3) présence de synapses secondaires, orientées longitudinalement, entre les cellules superficielles corticales contiguës; 4) cellules corticales superficielles non proéminentes, même à l'apex des rameaux; 5) taille des cellules superficielles corticales changeant peu depuis l'apex des rameaux jusque vers des zones plus proximales; 6) présence d'épaississements lenticulaires dans les parois des cellules médullaires; 7) tétrasporocystes arrangés parallèlement; 8) cystocarpes en forme de fiole. Cette espèce est aussi caractérisée par la production de C15 acétogénine 12-*epi*-obtusényne et du diterpénoïde déoxyparguéroïl 16-acétate (15-bromo-2,16-diacétoxy-7-hydroxy-9(11)-parguérène) comme métabolites secondaires halogénés.

KEY WORDS: Algae, Rhodophyta, Ceramiales, *Laurencia lageniformis*, chemotaxonomy, halogenated secondary metabolite.

INTRODUCTION

Our continuing studies on the morphology and halogenated compounds of species belonging to the red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) have established the taxonomic usefulness of secondary halogenated metabolites in combination with morphological features (Masuda *et al.*, 1996, 1997b). Such metabolites have been reported in genera of Bonnemaisoniales, Corallinales, Gigartinales, Rhodymeniales and Ceramiales among the Rhodophyta (Fenical, 1975). The Rhodomelaceae is known to be the most prolific in their synthesis of halogenated metabolites (Fenical, 1975). *Laurencia* produces more than 250 diverse metabolites that are classified into four groups: sesquiterpenoid, diterpenoid, triterpenoid, and C₁₅ acetogenin (Erickson, 1983). *Laurencia* species producing halogenated secondary metabolites are primarily included in the subgenus *Laurencia* defined by Saito (1967). However, some species apparently belonging to the subgenus *Chondrophyucus* Tokida *et* Saito (*in* Saito, 1967) have been also reported to produce such metabolites (Erickson, 1983). Each species of *Laurencia* that produces halogenated secondary metabolites can be discriminated by a characteristic set of such compounds. For example, *L. okamurae* Yamada and *L. composita* Yamada, two apparently closely related species which are morphologically very similar, have been shown to consistently differ with respect to the secondary compounds that they produce (Masuda *et al.*, 1996).

In Vietnamese waters, 20 species of *Laurencia* have been reported (Dawson, 1954; Pham, 1969; Nguyen *et al.*, 1993; Masuda *et al.*, 1997a, b, c). A further undescribed species of *Laurencia* was collected during our Vietnamese expedition of 1992/1993. In this paper, we describe it as a new species which has a characteristic set of morphological features and halogenated secondary metabolites.

MATERIALS AND METHODS

Specimens examined were collected at Mui Nai (5.ii.1993) and Bai No (6.ii.1993), Hatien, Kien Giang Province, Vietnam. For morphological study, specimens were initially fixed in 4% formalin in seawater, with some later dried as herbarium specimens. Sections were made by hand using a razor blade and pith stick, stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1:1:1:1) solution, and mounted in 50% glycerol-seawater on microscope slides. Voucher specimens are deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP 062087-062091).

Material for chemical analysis collected at Mui Nai was air-dried at room temperature (about 25°C) for a day. A partially-dried sample (84 g) was extracted with methanol and the solution concentrated *in vacuo*. The residue was partitioned between ether and water, with the ether solution shaken with water, dried over anhydrous Na₂SO₄, and evaporated to give an oily extract (1.23 g). The methanol extract (300 mg) was fractionated by column chromatography on silica gel (Merck, Kieselgel 60, 70-230 mesh). The fraction (70.3 mg) eluted with hexane/ethyl acetate (10:1) was then subjected to preparative TLC (Merck, Kieselgel 60F₂₅₄) with hexane/ethyl acetate (5:1) to afford crude "compound 1". This was then purified by preparative TLC with toluene to give pure "1" (25.0 mg; 8.3% of the extract). The fraction (45.4 mg) eluted with hexane/ethyl acetate

(1:1) was further chromatographed by preparative TLC with hexane/ethyl acetate (3:2) to give "compound 2" (5.8 mg; 1.9%). Identification of these compounds was carried out by detailed spectral analysis and comparison of the spectral data and $[\alpha]_D^{25}$ values was made with those reported in the literature.

RESULTS

Laurencia lageniformis Masuda, sp. nov.

Plantae ex axibus rectis multis utrinque e disco basali communi et e ramis stoloniformibus effecti constantes; thalli flavido-brunnei vel brunneo-lavandulae, aliquantum molles, exsiccatione chartae adherentes; axes principales 4-8 cm in longitudine, omnino teretes, percurrentes, usque ad 900 μ m in diametro, ramos plerumque oppositum in plano fere singulari interdum verticillatim vel alternatim ferentes; cellula axialis omnis cum cellulis periaxialibus quattuor, foveae-colligationes secundariae longitudinaliter dispositae inter cellulas corticales superficiales contingentes semper adsunt; cellulae corticales superficiales etiam prope apices ramorum non procurrentes, in sectionibus transversalibus ramuli nec radiatim elongatae nec in vallem dispositae; incrassationes lenticulares in parietibus cellularum medullae adsunt; tetrasporangia e cellulis periaxialibus in ramis ultimis et penultimis in successione acropetali formata, igitur in ordinatione parallela ad axem longitudinalem disposita; tetrasporangia matura 120-160 μ m in diametro; cystocarpia laterales in ramis, lageniformia, 600-900 μ m alta, 530-780 μ m lata, collo protrudente 100-250 μ m in longitudine; spermatangia non inventa.

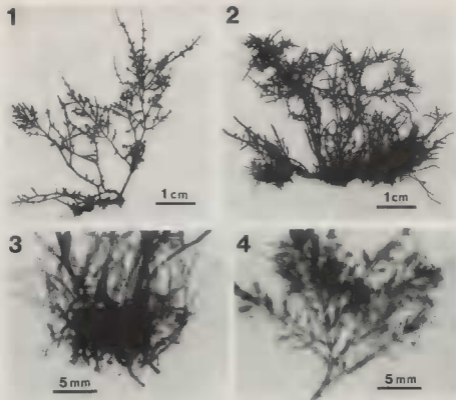
Plants consisting of many upright axes arising both from a common discoid holdfast and from stolon-like branches; thalli yellowish-brown or brownish-purple, somewhat soft, adhering to paper on drying; main axes 4-8 cm in length, terete throughout, percurrent, up to 900 μ m in diameter, bearing branches mostly oppositely in a nearly single plane sometimes verticillately or alternately; each axial cell with four periaxial cells; longitudinally oriented secondary pit-connections always present between contiguous superficial cortical cells; superficial cortical cells not projecting even at apices of branches, in transverse sections of branchlets neither elongated radially nor arranged as a palisade; lenticular thickenings present in the walls of medullary cells; tetrasporangia formed from periaxial cells on ultimate and penultimate branches in acropetal succession, therefore arranged parallel to the longitudinal axis; mature tetrasporangia 120-160 μ m in diameter; cystocarps lateral on branches, flask-shaped, 600-900 μ m high, 530-780 μ m wide with a protruding neck 100-250 μ m in length; spermatangia not found.

Holotype and type locality: SAP 062090 (Fig. 1), collected by M. Masuda on 5 February 1993, at Mui Nai, Hatien, Kien Giang Province, Vietnam.

Distribution: Endemic to southern Vietnam; known from Hatien, facing the Gulf of Thailand, and from Vung Tau (Pham, 1969, as *L. nidifica* J. Agardh), facing the South China Sea.

Etymology: The specific epithet, *lageniformis* (meaning flask-shaped) refers to the characteristic shape of the cystocarps.

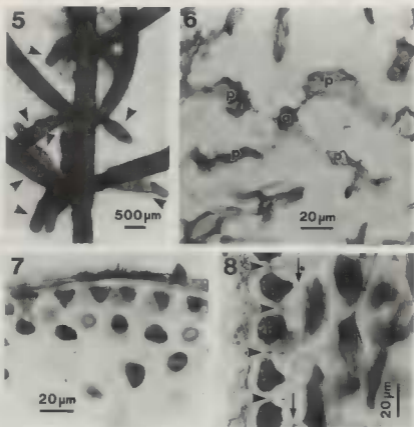
Plants grow on rocks or shellfish in the mid-intertidal zone of wave-exposed coasts. Some 20-40 upright axes (Fig. 2) arise from a basal system with numerous stolon-like lower branches and descending adventitious branchlets (Fig. 3). The basal systems of adjacent thalli are frequently entangled with each other, making it often difficult to distinguish individual plants. Each upright axis is percurrent, terete, and bears



Figs 1-4. *Laurencia lageniformis*. Mui Nai, Hatien, Kien Giang Province, Vietnam. Fig. 1. Portion of the cystocarpic specimen (holotype, SAP 062090). Fig. 2. Tetrasporangial specimen (SAP 062088). Fig. 3. Wet habit of the lowest portion of a plant, showing the fibrous basal system. Fig. 4. Wet habit of the middle portion of a plant, showing alternate, opposite and verticillate branches.

up to four orders of progressively shorter laterals. First-order branches are formed at intervals of 2-4 mm and at angles of 30-50°. Branches of all order are arranged mostly in a distichous-opposite manner. However, this branching is sometimes interrupted by either the addition of one or more branches or an elimination of one of the paired branches (Fig. 4); the original branching plane can also change slightly, resulting in a polystichous arrangement of branches. Main axes are 400-600 μm in diameter in proximal portions, 600-900 μm in lower to middle portions, and then taper gradually to 500-550 μm at the tips.

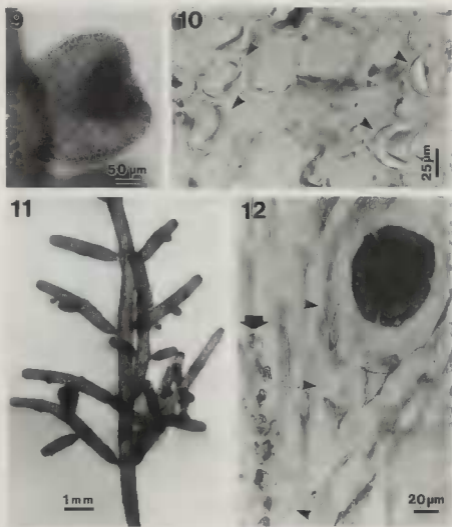
First-order branches are 2-4 cm long in the lower to middle portions of main axes and become progressively shorter above. Adventitious branches 1-10 mm long develop chiefly near the branched portions of main axes and the lower parts of first-order laterals at various angles (Fig. 5). The majority of these bear reproductive structures.



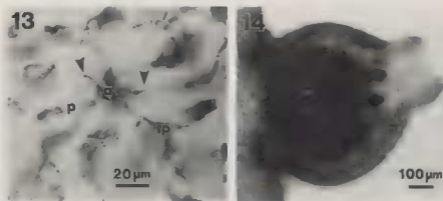
Figs 5-8. *Laurencia lageniformis*. Mui Nai, Hatien, Kien Giang Province, Vietnam. Fig. 5. Middle portion of a plant with adventitious branchlets (arrowheads) at various directions. Fig. 6. Transverse section (TS) of the upper portion of a second-order branch showing an axial cell (a) with four periaxial cells (p). Fig. 7. TS of the upper portion of a second-order branch. Fig. 8. Longitudinal section (LS) of the lower portion of a first-order branch showing longitudinally oriented secondary pit-connections (arrowheads) between contiguous superficial cortical cells; arrows indicate primary pit-connections.

The growing point is always sunk in an apical pit, as is typical of the genus. Axial cells are recognizable only just behind the apical cell, but each produces four periaxial cells (Fig. 6). Superficial cortical cells of distal parts of branches of all orders are polygonal, 8-16 μm long by 10-24 μm wide (a length:width ratio of 0.4-1.0), and are regularly arranged in longitudinal rows in surface view. Superficial cortical cells of proximal parts of well-developed, first-order laterals are 18-36 μm long by 16-36 μm wide (a length:width ratio of 0.6-2.0).

Superficial cortical cells in transverse section are 12-18 μm thick in upper portions of first-order branches and 22-30 μm thick in proximal portions. Because of their



Figs 9-12. *Laurencia lageniformis*. Mui Nai, Hatien, Kien Giang Province, Vietnam. Fig. 9. Smooth contours of a young third-order branchlet imparted by the non-projecting superficial cortical cells. Fig. 10. TS of medullary cells in the lower portion of a first-order branch, many with lenticular thickenings (arrowheads). Fig. 11. Distal portion of a second-order branch with tetrasporangial branchlets. Fig. 12. LS of a tetrasporangial branch; wide arrow indicates a row of axial cells, and three arrowheads indicate an elongated, fertile periaxial cell.



Figs 13, 14. *Laurencia lageniformis*. Mui Nai, Hatien, Kien Giang Province, Vietnam. Fig. 13. TS of a tetrasporangial branch showing an axial cell (a) with thick vegetative periaxial cells (p) and two probable fertile periaxial cells (arrowheads). Fig. 14. Rostrate cystocarp.

obconical profiles, they do not form a palisade layer (Fig. 7). Longitudinally oriented secondary pit-connections are present between contiguous superficial cortical cells (Fig. 8), the cells never projecting even at branch apices (Fig. 9). Lenticular thickenings 8-20 µm thick are present in the walls up to 2 µm thick of medullary cells (Fig. 10) except in distal portions of branches. Their frequency, however, is variable as some branches have few thickenings whereas others have many (Fig. 10). Medullary cells are subisodiametric and up to 80 µm in diameter in middle to lower portions of first-order branches. As living material was not available, examination of *corps en cerise* was not performed.

Tetrasporangia are formed within ultimate and penultimate ordinary (Fig. 11) and adventitious branches that reach 1-5 mm long by 350-500 µm wide. The tetrasporangial initial is cut off abaxially from an elongated periaxial cell (Fig. 12). Only two of the four periaxial cells elongate and produce tetrasporangia in each fertile tier (Fig. 13). Each tetrasporangium is provided with two cover cells¹ that are distally produced by the fertile periaxial cell (Fig. 12). Tetrasporangia mature acropetally, and young to almost mature sporangia are in a parallel arrangement relative to the longitudinal axis of the branch (Fig. 11). Mature tetrasporangia are 120-160 µm in diameter.

Cystocarps are borne laterally on the second- to fourth-order branches. Mature cystocarps are flask-shaped (Fig. 14) and 600-900 µm high by 530-780 µm wide. They have necks 100-250 µm long and flared ostiolar rims 200-400 µm wide.

The major halogenated metabolite, "compound 1" was identified as the C₁₅ acetogenin 12-*epi*-obtusenyne by independent structural elucidation, as well as detailed comparison of spectral data and optical rotation with those reported in the literature

1. Nam & Saito (1990) use the terms "pre-" and "post-sporangial cover cells". Post-sporangial cover cells, however, divide to form cortical filaments as do derivatives of sterile periaxial cells (Nam & Saito, 1990), rather than persist as special cells covering the tetrasporangia. We therefore reserve the term "cover cells" for pre-sporangial cover cells (Masuda *et al.*, 1996), as only these cells remain undivided and are specifically associated with tetrasporangia.

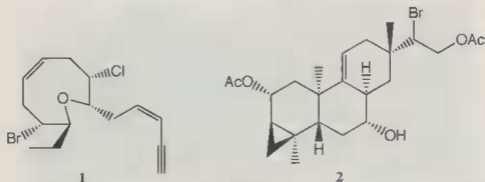


Fig. 15. Molecular structures of secondary metabolites from *Laurencia lageniformis*. 1. 12-*epi*-obtusenyne; 2. deoxyparguerol 16-acetate (15-bromo-2,16-diacetoxy-7-hydroxy-9(11)-parguerene).

(Gopichand *et al.*, 1981). Another metabolite, "compound 2" was identified as the diterpenoid deoxyparguerol 16-acetate (15-bromo-2,16-diacetoxy-7-hydroxy-9(11)-parguerene) (Higgs & Faulkner, 1982). The structures of identified metabolites are shown in Figure 15.

DISCUSSION

Laurencia lageniformis is primarily characterised by flask-shaped (rostrate) cystocarps comparable to those of the western Pacific species *L. capituliformis* Yamada (Saito, 1967), *L. cartilaginea* Yamada (Nam & Saito, 1990), *L. papillosa* (C. Agardh) Greville (Cribb, 1958; Masuda *et al.*, 1997a), *L. tranoi* Ganzon-Fortes (1982) and *L. palisada* Yamada (unpublished observations on plants from Ilocos Norte, Luzon Island, Philippines; 20.vi.1990, leg. M. Masuda, SAP 062480, 062481). These five species all differ from *L. lageniformis*, however, in lacking longitudinally oriented secondary pit-connections between contiguous superficial cortical cells (Saito, 1967, 1969; Ganzon-Fortes, 1982; Masuda, unpublished observations).

An Australian species, *Laurencia forsteri* (Mertens *ex* Turner) Greville, has longitudinally oriented secondary pit-connections between contiguous superficial cortical cells as well as cystocarps with an inconspicuous to prominent neck (Saito & Womersley, 1974), but it differs from *L. lageniformis* in the absence of stolon-like lower branches and its non-opposite branches (Saito & Womersley, 1974). A Pacific North American species, *Laurencia masonii* Setchell *et* Gardner, possesses urceolate cystocarps with a prominent rostrum and stolon-like basal laterals (Dawson, 1963), but differs from *L. lageniformis* in its essentially larger (6-20 cm high) and thicker (the axes being 1.5-3.5 mm wide) thalli as well as its branching (Dawson, 1963). *Laurencia coelenterata* Ballantine *et* Aponte (1995), recently described from the Dry Tortugas, Florida, has flask-shaped cystocarps but differs from *L. lageniformis* in having diminutive thalli up to 12 mm in height that arise from a

basal crust and tetrasporangia formed in a perpendicular arrangement to the longitudinal axes of the bearing branchlets.

Laurencia lageniformis is strongly characterised by its mostly distichous-opposite branching, although it may be verticillate or alternate in some instances. Four basic types of branching pattern are known in species of the genus: 1) distichous, 2) unilateral, 3) spiral, and 4) verticillate. The distichous type occurs in many species with compressed to flattened axes. The unilateral type is rare and primarily found in species with creeping axes such as *L. articulata* Tseng, *L. decumbens* Kützinger and *L. perforata* (Bory) Montagne (Kützinger, 1865; Tseng, 1943; Saito, 1969; Cribb, 1983; McDermid, 1988). The spiral type is common in species with upright, terete axes, including *L. forsteri* (Saito & Womersley, 1974) and *L. masonii* (Dawson, 1963). The verticillate type is extremely rare. *Laurencia verticillata* Zhang et B. M. Xia (1980) from Xisha Islands, southern China, was reported to show characteristic verticillate branching throughout the thallus. The Southern Australian *Laurencia clavata* Sonder also shows verticillate branching (Saito & Womersley, 1974). According to Cribb (1983), *L. venusta* Yamada from the southern Great Barrier Reef commonly has verticillate branches, although branching can also be alternate or opposite. Re-examination of the holotype specimen of *L. venusta* collected at Shimokoshi-jima, Kagoshima Prefecture, in July 1918 (SAP 013873) revealed that it has both verticillate and irregular branching. Thus, the spiral branching is irregular, and its close intervals may bear partially verticillate or opposite branching. A similar irregular branching pattern has been reported in many terete species of *Laurencia* (Yamada, 1931; Cribb, 1958, 1983; Saito, 1967). However, the branching of *L. lageniformis* seems to be basically distichous-opposite, this pattern being occasionally interrupted by the addition of one or more branches (i.e., verticillate) or lack of one of the normally paired opposite branches (i.e., alternate).

Cribb (1958, p. 160) has pointed out the taxonomic usefulness of superficial cortical cells of *Laurencia* and made the following comments: "Size and shape of surface cells in the main branches is variable in some species, but in others, such as *L. tenera* and *L. flagellifera*, this character is a constant diagnostic feature". Where the cells are measured seems to be critical to their potential taxonomic usefulness. According to Saito & Womersley (1974), superficial cortical ("epidermal") cells are small near branch apices and become wider and longer below in many Southern Australian species, although those of a few species such as *L. clavata*, *L. elata* (C. Agardh) Hooker et Harvey and *L. patentiramea* (Montagne) Kützinger² (as *L. paniculata* (C. Agardh) J. Agardh) never elongate. The lengths and widths of superficial cortical cells of *L. lageniformis* change only slightly from the apices to the bases of branches, a further defining feature of this species.

The Hawaiian *Laurencia nidifica* J. Agardh is somewhat similar to *L. lageniformis* in having opposite, verticillate, or alternate branches (J. Agardh, 1863). Its lectotype specimen, designated by Yamada (1931, p. 202), has been shown by McDermid (1988, fig. 20) to bear a faint resemblance to our Vietnamese specimens, although it has ovate-spherical cystocarps (J. Agardh, 1863). The alga reported by Pham (1969) from Vietnam as *L. nidifica* actually seems better referable to *L. lageniformis* on the basis of his illustrations

2. According to Silva et al. (1996), the correct name of *Laurencia patentiramea* and *L. paniculata* should be *Laurencia glandulifera* (Kützinger) Kützinger. However, Saito (1985), who examined the type material and liquid-preserved specimens of *L. glandulifera* from Adriatic Sea, reported the presence of longitudinally oriented secondary pit-connections and parallel arrangement of tetrasporangia. These features are entirely different from those of the alga passing under the name *L. paniculata* (Saito & Womersley, 1974). Further critical studies are clearly needed to elucidate the taxonomic status of these species.

showing a distichous-opposite branching (Pham, 1969, fig. 2.215). *Laurencia nidifica* reported by Cribb (1958) from Queensland, Australia, has ovoid to urceolate cystocarps, and its gross morphology (Cribb, 1958, pl. 5, fig. 12, pl. 6, figs 1-3) is similar to that of *L. lageniformis*. A further critical study is needed to ascertain the status of this Australian alga that is probably referable to *L. lageniformis* rather than *L. nidifica*.

Laurencia lageniformis is characterised by a set of halogenated secondary metabolites: the C₁₅ acetogenin 12-*epi*-obtusenyne and the diterpenoid deoxyparguerol 16-acetate (15-bromo-2,16-diacetoxy-7-hydroxy-9(11)-parguerene). 12-*epi*-obtusenyne has previously been isolated from the sea hare *Aplysia dactylomela* Rang collected at Bimini, Bahamas (Gopichand *et al.*, 1981), indicating that *Laurencia* species producing this compound are probably present at that locality. Deoxyparguerol 16-acetate has previously been isolated from *Laurencia obtusa* (Hudson) Lamouroux collected at Kimberidge Bay, Dorset (Higgs & Faulkner, 1982). Moreover, deoxyparguerol, which is the parent alcohol of deoxyparguerol 16-acetate, has also been isolated from the same *Aplysia* species collected from off the coast of La Parguera, Puerto Rico (Schmitz *et al.*, 1982). These results also suggest that the Caribbean sea hare *A. dactylomela* consumes *Laurencia* species (Erickson, 1983).

Laurencia lageniformis differs from *L. nidifica* in the aforementioned compounds. *Laurencia nidifica* in Hawaii has been reported to include three chemically distinct populations, each producing unrelated sets of halogenated secondary metabolites that are entirely different from those of *L. lageniformis* (Erickson, 1983). The Hawaiian *L. nidifica* includes two pink populations, one elaborating cyclolaurane- and laurane-type sesquiterpenoids (Waraszkiewicz & Erickson, 1974), the other elaborating chamigrane-type sesquiterpenoids (Waraszkiewicz & Erickson, 1974, 1975, 1976; Waraszkiewicz *et al.*, 1977), and a green population containing C₁₅ acetogenins with unusual carbocyclic skeletons (Sun *et al.*, 1976; Waraszkiewicz *et al.*, 1976, 1978). Re-examination of morphological features of these three populations of *L. nidifica* is clearly needed.

The occurrence of four periaxial cells from each axial cell is a critical feature that assigns *L. lageniformis* to the subgenus *Laurencia* (Nam & Saito, 1995), a group of species in which the production of halogenated secondary metabolites may be a particularly good taxonomic feature (Masuda *et al.*, 1997b). *Laurencia palisada* Yamada and *L. perforata* (Bory) Montagne of the subgenus *Chondrophycus* have been reported to produce some characteristic halogenated metabolites (Paul & Fenical, 1980; Gonzalez *et al.*, 1977). The identification, however, is questionable (Masuda *et al.*, 1997c, and unpublished data).

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